

# Diverse Macular Dystrophy Phenotype Caused by a Novel Complex Mutation in the *ELOVL4* Gene

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**PURPOSE.** A 5-bp deletion in *ELOVL4*, a photoreceptor-specific gene, has been associated with autosomal dominant (ad) macular dystrophy phenotypes in five related families, in which phenotypes range from Stargardt-like macular dystrophy (STGD3; Mendelian Inheritance in Man 600110) to pattern dystrophy. This has been the only mutation identified in *ELOVL4* to date, which is associated with macular dystrophy phenotypes. In the current study, the potential involvement was investigated of an *ELOVL4* gene variation in adSTGD-like and other macular dystrophy phenotypes segregating in a large unrelated pedigree from Utah (K4175).

**METHODS.** The entire open reading frame of the *ELOVL4* gene was analyzed by direct sequencing in a proband from the K4175 family. The combination of denaturing high-performance liquid chromatography (DHPLC) analysis and direct sequencing of all available family members was used to further assess segregation of identified *ELOVL4* variants in the pedigree.

**RESULTS.** A complex mutation, two 1-bp deletions separated by four nucleotides, was detected in all affected members of the family. The mutation results in a frameshift and the truncation of the *ELOVL4* protein, similar to the effect of the previously described 5-bp deletion.

**CONCLUSIONS.** The discovery of a second mutation in the *ELOVL4* gene segregating with macular dystrophy phenotypes confirms the role of this gene in a subset of dominant macular dystrophies with a wide range of clinical expressions and suggests a role for modifying genes and/or environmental factors in the disease process. (*Invest Ophthalmol Vis Sci.* 2001; 42:3331-3336)

Stargardt macular dystrophies are a collection of early-onset disorders characterized by macular atrophy surrounded by lipofuscin-containing orange-yellow fundus

flecks. Stargardt disease (STGD; Mendelian Inheritance in Man [MIM] 248200) can be inherited as an autosomal recessive (ar) trait (MIM 248200) or an autosomal dominant (ad) trait (MIM 600110; 603786). All recessive forms of STGD have been linked to a locus on 1p32, containing the *ABCR* (*ABCA4*) gene.<sup>1</sup> Mutations in *ABCR*, a photoreceptor-specific adenosine triphosphate (ATP)-binding cassette (ABC) transporter, are causal in arSTGD<sup>2</sup> and in a number of other retinal diseases, such as recessive cone-rod dystrophy (CRD),<sup>3</sup> and some forms of autosomal recessive retinitis pigmentosa (RP19).<sup>4,5</sup> Heterozygous carriers of *ABCR* variant alleles are more susceptible to age-related macular degeneration (AMD), a late-onset complex trait.<sup>6,7</sup> Rare autosomal dominant forms of STGD-like phenotypes have been mapped to several loci on the human genome, including those on chromosome 6q14 (STGD3)<sup>8</sup> and 4p (STGD4).<sup>9</sup> Recently, we cloned and characterized the gene responsible for STGD-like macular dystrophy (STGD3) on 6q14.<sup>10</sup> The protein, encoded by this photoreceptor-specific gene, elongation factor of very-long-chain fatty acids (*ELOVL4*), probably performs an important function in the biosynthesis of photoreceptor outer segment membrane components. Affected members in all five families segregating the STGD3 or other macular dystrophy phenotypes harbored the same presumed founder mutation, 797-801delAACTT.<sup>10</sup> This 5-bp deletion causes a frameshift and premature termination of the protein, strongly suggesting the pathogenic nature of this sequence change. Until now, screening of patients with AMD or additional families with autosomal dominant macular dystrophy phenotypes has not revealed any other disease-associated *ELOVL4* sequence variants<sup>11</sup> to directly support the previous conclusion. In the current study, we investigated the potential involvement of *ELOVL4* gene variation in adSTGD-like and other macular dystrophy phenotypes segregating in a large pedigree from Utah.

## MATERIALS AND METHODS

### Recruitment of Subjects

A large family with apparent autosomal dominant macular dystrophy was identified from the clinic population of the Moran Eye Center of the University of Utah. Complete eye examinations, including best corrected visual acuity, dilated funduscopy, and color fundus photography were performed, and blood was collected from all available family members who were able to come to Salt Lake City. Selected individuals had digital fluorescein angiograms recorded (Imagenet; Topcon Optical, Tokyo, Japan) system. Family members unable to come to Salt Lake City had examinations, photography, and blood collection performed by their local ophthalmologists. All participants signed institutionally approved consent forms, and recruitment and research procedures complied with the tenets of the Declaration of Helsinki.

### Mutation Detection by Direct Sequencing

DNA samples from two first-cousin family members (III-1 and III-7) were analyzed for *ELOVL4* variants by direct sequencing. Each exon of

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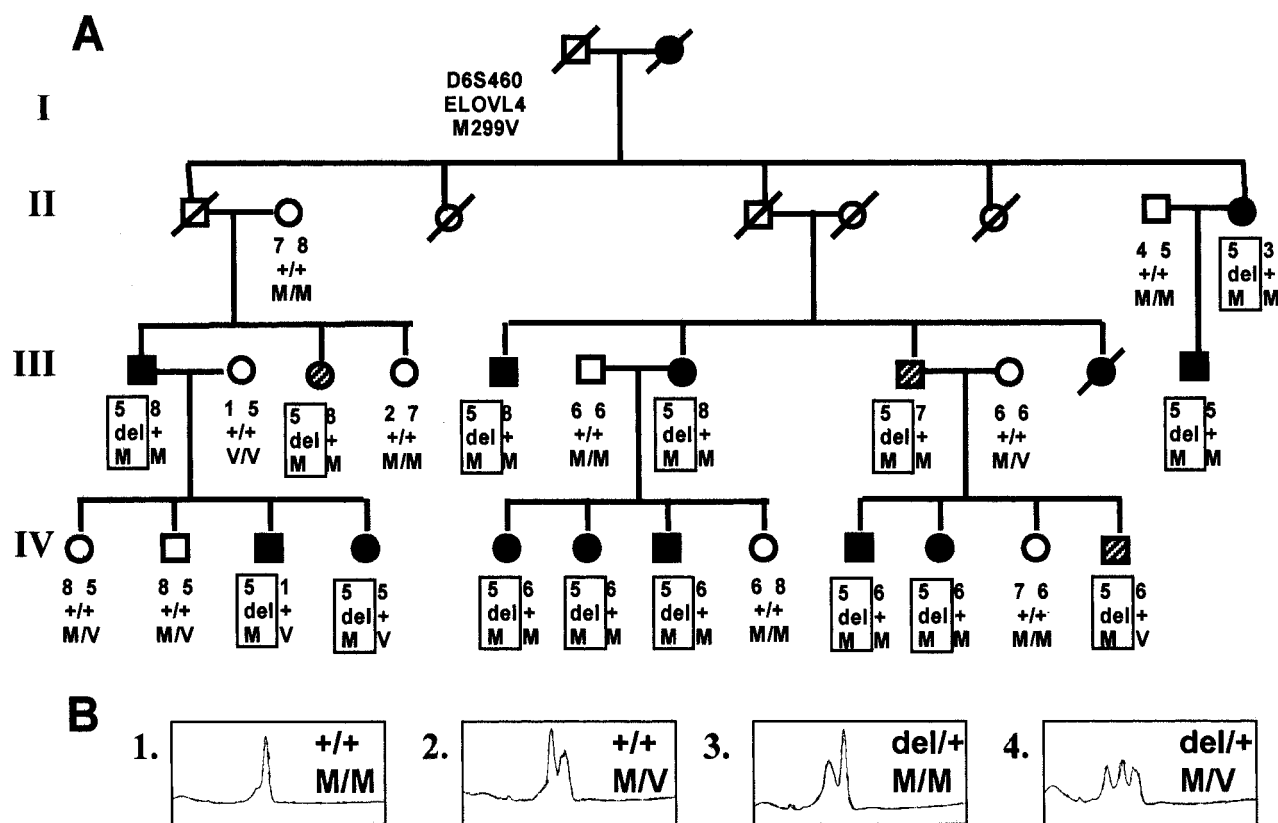
Supported by Grants EY-11600, EY-11309, and EY-13435 from the National Institutes of Health, Bethesda, Maryland; by grants from the Foundation Fighting Blindness and Research to Prevent Blindness; and by the Steinbach Fund, New York, New York.

Submitted for publication June 1, 2001; revised August 22, 2001; accepted September 5, 2001.

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**FIGURE 1.** K4175 pedigree and segregation of genotypes. (A) Filled circles and squares: affected females and males, respectively; stippled circles and squares: nonpenetrant females and males, respectively; open circles and squares: unaffected females and males, respectively. Below each individual are genotypes as determined by the dinucleotide marker D6S460 and the combination of DHPLC and direct-sequencing information from the *ELOVL4* locus for the two, single-nucleotide deletions (*ELOVL4*del) or wild type (+) and the methionine (M) to valine (V) polymorphism (M299V) in exon 6 of *ELOVL4*. The disease chromosome is boxed. Affected status of deceased individuals was determined by reports from living family members. (B) DHPLC profiles for the four possible combinations of *ELOVL4* alleles in exon 6. The homozygous +/+; V/V genotype in individual III-2 is indistinguishable from the wild-type +/+; M/M by DHPLC and so was determined by direct sequencing.

the *ELOVL4* gene was amplified by PCR with previously described primers,<sup>10</sup> and sequencing was performed according to the manufacturer's protocols (Model ABI 377; PE Biosystems, Foster City, CA). Similarly, all exons of the *ABCA4* and *peripherin/RDS* genes were directly sequenced with previously published primer pairs.<sup>2</sup>

### Subcloning of *ELOVL4* Alleles

The sequence of mutant *ELOVL4* alleles was determined directly from the chromatograms of heterozygous samples. To further demonstrate the exact sequence of individual *ELOVL4* alleles in exon 6 in patient III-1, the gel-purified PCR product was cloned into pCR2.1-TOPO vector using a cloning system (Topo TA Cloning; Invitrogen, San Diego, CA) according to the manufacturer's protocol. Twelve individual clones were picked for sequencing, which was performed as described earlier.

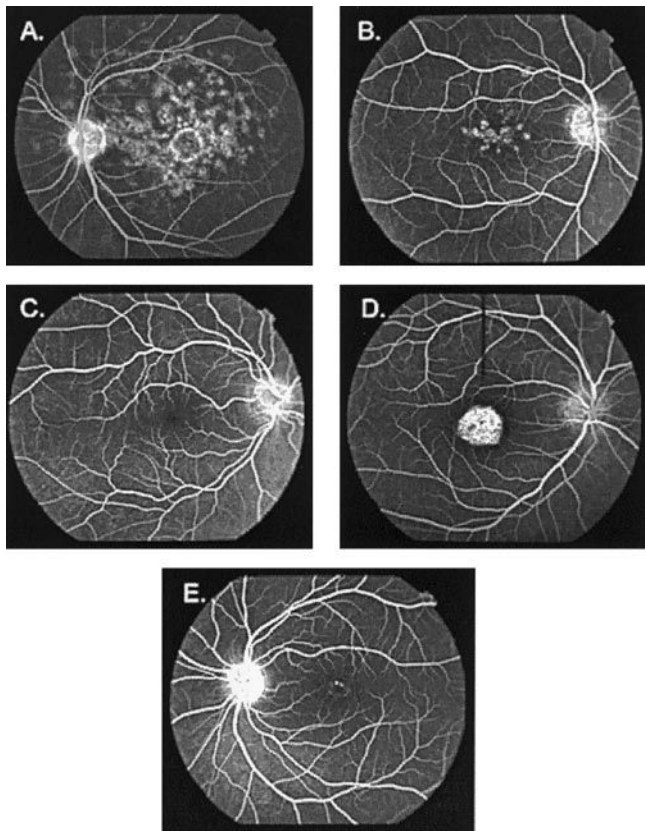
### Segregation Analysis by Denaturing High-Performance Liquid Chromatography

DNA of all available members of the K4175 pedigree was PCR amplified with specific primers for exon 6 of *ELOVL4*.<sup>10</sup> After the PCR reaction, heteroduplex DNA formation was achieved by heating the samples to 95°C for 2 minutes and then lowering the temperature 1°C per minute until 60°C was reached. Samples were separated on an HPLC system (Helix; Varian Instruments, Walnut Creek, CA), using the standard running program supplied by the manufacturer. Samples were grouped according to the elution profile. In addition, exon 6 was directly sequenced in every member of the K4175 pedigree, confirming the grouping and the exact genotype. To assess the frequency of *ELOVL4* mutant alleles in the general population, the same DHPLC

analysis was performed for exon 6 of *ELOVL4* on 292 healthy control individuals and on 513 patients with diagnosed AMD. One DNA sample from each group was included on every run as a positive control (a representative of a specific genotype). If a sample deviated from any of the controls, indicating a different genotype, it was directly sequenced as described earlier.

### Linkage Analysis

DNA was isolated from venous blood as has been described. For linkage analysis, DNA was amplified under the following conditions: 100 to 200 ng DNA, 1.5 mM Mg PCR buffer, 200 μM dNTPs, 4% dimethyl sulfoxide (DMSO), 10 pmol primer, (forward primer end-labeled with γ-ATP, using T4 polynucleotide kinase; Molecular Biology Resources, Milwaukee, WI), and 1 U *Taq* polymerase (Roche Molecular Biochemicals, Indianapolis, IN), in a 25-μl reaction at 94°C for 5 minutes; 8 cycles of 94°C for 20 seconds, 64°C for 20 seconds less 1°/cycle, and 72°C for 40 seconds; followed by 23 cycles of 94°C for 20 seconds, 56°C for 20 seconds, 72°C for 40 seconds, and a final extension of 72°C for 10 minutes. The resultant PCR products were electrophoresed on 7% polyacrylamide gels for 3.5 hours. The polymorphic marker D6S460 was genotyped in all 25 (12 affected and 13 unaffected) individuals, and two-point linkage analysis was performed with the LINKAGE program (provided in the public domain by the Human Genome Mapping Project Resources Center, Cambridge UK, and available at <http://www.hgmp.mrc.ac.uk>) under assumptions of a dominant model, a penetrance of 0.7, and a disease gene frequency of 0.001. Primer sequence and heterozygosity for this marker can be obtained at <http://gdbwww.gdb.org/gdb/gdbtop.html>.



**FIGURE 2.** Early-phase fluorescein angiograms from representative individuals in kindred 4175 carrying *ELOVL4* deletions. (A) Foveal atrophy with extensive flecks (III-1); (B) butterfly-pattern dystrophy of the central macula without flecks (III-7); (C) normal fluorescein angiogram (III-8); (D) beaten-metal-appearing macular lesion without flecks (IV-3); and (E) subtle bull's-eye hyperfluorescence around the fovea without flecks (IV-10). The absence of a dark choroid was noted in all individuals.

## RESULTS

### Phenotypic Evaluation of Patients

We identified a large Utah kindred from the clinic population of the Moran Eye Center with autosomal dominant macular dystrophy (Fig. 1). In this family, phenotypes ranged from STGD3 to butterfly-pattern dystrophy<sup>12</sup> to bull's-eye maculopathy (Fig. 2). Twenty-five family members from 10 to 68 years of age were examined ophthalmoscopically, and blood samples were obtained for genetic analyses. Twelve family members exhibited varying degrees of maculopathy with impaired visual acuity (Table 1). The remaining 13 had completely normal results in fundus examinations and no evidence of significant visual loss. The most common age of onset of symptoms was in the second decade of life, although it varied widely, from as early as 6 years of age (IV-7), to as late as 63 (individual II-8). The disease in the latter patient had initially been diagnosed by her local ophthalmologist as AMD, but the diagnosis was later changed because of the obvious presence of typical Stargardt-like flecks rather than AMD-associated soft and hard drusen. Patients reported blurred central vision, but no nyctalopia, loss of visual field, or photophobia. Five patients were considered legally blind (vision 20/200 or worse, bilaterally). At the same time, several other affected members of the family were still able to drive and perform job functions.

### Deleterious Mutation in the *ELOVL4* Gene

The combination of DHPLC analysis and direct sequencing of all family members revealed a rare, complex mutation in all

those affected (Fig. 1). The mutation can be described as a deletion of two nucleotides, four nucleotides apart, in exon 6 of the *ELOVL4* gene (Fig. 3). It was interesting that this mutation occurred in the same location as the previously described 5-bp deletion and had exactly the same truncating effect on the *ELOVL4* protein: deletion of the last 51 amino acids, including the dilysine-targeting signal (Fig. 3). Occurrence of the mutation in all affected members of the family and the premature termination of the protein allow us to suggest the causal role of this mutation. Additional proof for this statement was gained from the large-scale analysis of healthy control individuals and patients with AMD. Not one of 292 general-population control subjects or 513 patients with AMD (a total of 1610 chromosomes) harbored any deleterious changes in exon 6 of *ELOVL4*.

In addition to the double deletion, the only other detected variant in the *ELOVL4* gene sequences in this family was the 973A → G (M299V) change in exon 6. This allele has been detected as the most frequent single nucleotide polymorphism (SNP) in the *ELOVL4* gene (allele frequency, ~0.1) in the general population and deemed not to be associated with any disease phenotype.<sup>10,11</sup> We did not find any evidence of segregation of this variant with the disease phenotype (or its variation) in this study (Fig. 1).

### Linkage Analysis

The mutation segregated in an autosomal dominant pattern with maculopathy in kindred 4175 (Fig. 1). To further confirm that the dominant disease locus in this kindred resides at the chromosome 6q14 location of the *ELOVL4* gene, we genotyped all family members with a highly informative microsatellite marker (D6S460) tightly linked to the *ELOVL4* locus. The marker D6S460 resides less than 0.6 centimorgans (cM) proximal to the *ELOVL4* locus and is the marker that has been completely linked to the disease gene in initial linkage studies<sup>8,13</sup> and is the proximal flanking marker published by Zhang et al.<sup>10</sup> In kindred 4175 a maximum two-point lod score of 3.86 was obtained with genotypes generated by this microsatellite marker at a recombination fraction of  $\theta = 0.001$ , providing additional, significant evidence of localization of the disease gene locus to the *ELOVL4* locus on chromosome 6q14.

### Penetrance of the *ELOVL4* Mutation

All affected members in kindred 4175 as well as three nonpenetrant individuals—III-3, III-8, and IV-12—carried the disease haplotype of allele 5 for the marker D6S460 and the two single-nucleotide deletions in the *ELOVL4* gene (Fig. 1). One of the three nonpenetrant individuals was a 10-year-old (IV-12), well below the average age of onset of symptoms in this family. However, his father (III-8) and one of the father's cousins (III-3) were both 47 years old, carried the deletions, and had completely normal findings in eye examinations (including normal fluorescein angiography for individual III-8; Fig. 2C). Yet both of these individuals have affected siblings, and III-8 has two affected children (Fig. 1). So far, we have been unable to identify any environmental or hereditary factors that determined the nonpenetrant status of these two adults. One of the many clinical manifestations of maculopathy segregating in the family could still develop in these individuals at a later age. A precedent for this in this family is the individual II-8, in whom the very late onset (at more than 60 years of age) caused an initial misdiagnosis (as AMD).

## DISCUSSION

The macular phenotype of the family described in this study was diverse and generally consistent with the clinical description of affected members of families with macular dystrophies linked to 6q14, most of whom are known to harbor the 5-bp



TABLE 1. Characteristics of Kindred K4175

Generation	No.	Age	Age of Onset	<i>ELOVL4</i> Deletions Present	<i>ELOVL4</i> M299V Present	Right Eye Acuity	Left Eye Acuity	Phenotype
II	2	74		No	No	NA	NA	Normal
II	7	70		No	No	20/25	20/25	Normal
II	8	68	63	Yes	No	20/400	20/400	Mild foveal pigment disruption with flecks.
III	1	51	8	Yes	No	20/200	20/200	Foveal atrophy with flecks
III	2	48		No	Yes*	20/20	20/25	Normal
III	3	47		Yes	No	NA	NA	Normal
III	4	44		No	No	NA	NA	Normal
III	5	50	13	Yes	No	20/70	20/50	"Butterfly" pattern dystrophy, no flecks
III	6	51		No	No	20/20	20/20	Normal
III	7	49	18	Yes	No	20/60	20/50	"Butterfly" pattern dystrophy, no flecks
III	8	47		Yes	No	20/20	20/15	Normal
III	9	43		No	Yes	20/15	20/15	Normal
III	11	33	27	Yes	No	20/80	20/80	Foveal pigment disruption with flecks
IV	1	27		No	Yes	20/15	20/20	Normal
IV	2	25		No	Yes	20/15	20/15	Normal
IV	3	23	13	Yes	Yes	20/80	20/70	"Beaten metal" macula, no flecks
IV	4	21	12	Yes	Yes	20/200	20/200	"Beaten metal" macula with flecks
IV	5	27	12	Yes	No	20/200	20/200	Foveal pigment disruption with flecks
IV	6	25	7	Yes	No	20/200	20/200	Foveal atrophy with flecks
IV	7	22	6	Yes	No	20/70	20/80	"Butterfly" pattern dystrophy, no flecks
IV	8	18		No	No	20/20	20/15	Normal
IV	9	21	16	Yes	No	20/100	20/100	Foveal pigment disruption, no flecks
IV	10	18	18	Yes	No	20/40	20/20	"Bulls-eye" fovea, no flecks
IV	11	16		No	No	20/20	20/20	Normal
IV	12	10		Yes	Yes	20/25	20/25	Normal

NA, documented visual acuity not available.  
\*Homozygous individual.

deletion in the *ELOVL4* gene.<sup>10,14</sup> The first two descriptions of families in which maculopathy was ultimately linked to the STGD3 locus on 6q14 emphasized the phenotypic similarity to STGD and fundus flavimaculatus, as evidenced by the presence of macular atrophic lesions and abundant midperipheral sub-retinal flecks.<sup>8,14</sup> Subsequent studies demonstrated that many

other forms of maculopathy coexist in these STGD3 kindreds. Lagali et al.<sup>15</sup> described a family with a variety of macular pathologies ranging from macular atrophy with flecks, to pattern dystrophy, to retinal pigment epithelium (RPE) defects in a ring-shaped pattern.<sup>15</sup> Likewise, Griesinger et al.<sup>16</sup> reported that many of the affected family members in their study had

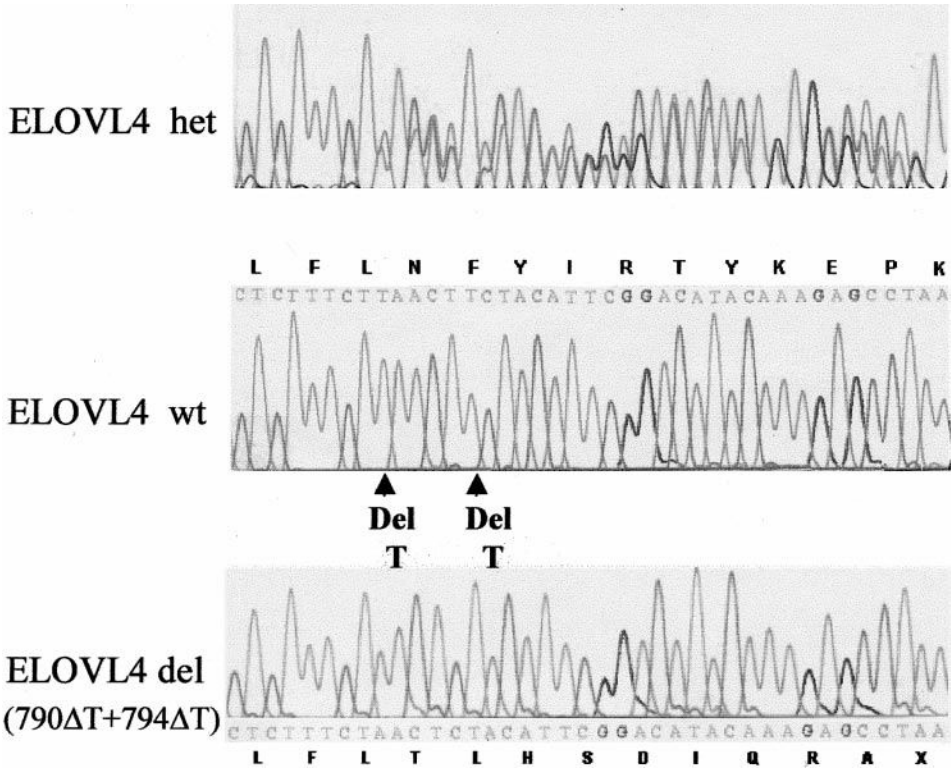


FIGURE 3. Complex deletion in the *ELOVL4* gene in patients from the K4175 pedigree. Partial sequence of exon 6 in patient III-1. After the initial sequence analysis determined the double deletion in exon 6 (*ELOVL4*het), the PCR product was cloned and alleles sequenced separately. The nucleotide sequences and protein translations are shown above and below the direct-sequencing traces. The double deletion causes a frameshift that results in a premature stop codon after 10 amino acids (*bottom trace*). *ELOVL4*wt, wild type allele; *ELOVL4*del (790ΔT+794ΔT), allele with the double deletion.

either central macular atrophy surrounded by flecks or ring-shaped RPE defects best seen with fluorescein angiography. A 10-generation STGD3-affected family described by Edwards et al.<sup>13</sup> includes one of the families reported to have the 5-bp deletion in *ELOVL4*.<sup>10</sup> In this very large family, affected members commonly had atrophic macular lesions, often with a "beaten-metal" appearance. Most individuals had extensive subretinal flecks in the midperipheral retina except in the early stages of the disorder. A few affected family members showed a pattern-dystrophy-like appearance or geographic atrophy of the posterior pole.

It should be noted that not one affected member of all these families ever exhibited a dark choroid on fluorescein angiograms—a feature often considered characteristic of arSTGD.<sup>12</sup> The choroidal silence of arSTGD is thought to be due to blocking of choroidal fluorescence by excessive deposition of lipofuscin in the RPE secondary to dysfunctional retinoid processing by *ABCR*.<sup>12</sup> The general absence of a dark choroid in *ELOVL4*-mediated autosomal dominant macular dystrophy implies that excessive lipofuscin deposition is not involved in this particular disease's pathogenesis, even though it shares many clinical similarities with arSTGD.

Some of the Utah family members exhibited macular changes indistinguishable from STGD (or fundus flavimaculatus), such as foveal atrophy or beaten-metal changes surrounded by deep flecks (Fig. 2A). Others had similar atrophic findings with no flecks (Fig. 2D). Three patients had deep yellowish lesions in the macula, characteristic of butterfly-pattern dystrophy (Fig. 2B). Still others (Fig. 2E) had mild to moderate pigmentary disruption of the fovea, sometimes in a bull's-eye pattern. None of the affected members had any abnormalities of the peripheral retina. Six affected family members (III-1, III-7, III-8, IV-3, IV-9, and IV-10) had fluorescein angiograms performed, but a dark choroid was never seen. The presence of flecks was strongly associated with poor visual acuity; only one person with flecks (III-11) had acuity better than 20/200. Conversely, only one person without flecks (IV-9) had acuity worse than 20/80.

The nature of the mutation found in the K4175 family is of specific interest. Similar mutations, involving noncontiguous deletions on the same allele, are extremely rare and have been described in only a few instances. For example, the Human Gene Mutation Database (Institute of Medical Genetics, Cardiff, UK; available at <http://archive.uwcm.ac.uk/uwcm/mg/hgmd0.html>) documents only a handful of mutations of similar nature. No like mutation, two 1-bp deletions close to each other, has been described in the literature. Single examples of mutations in three genes, *ATM*, *FBN1*, and *HR*, in which two deletions occur in proximity (5–15 bp) on the same allele, have been described.<sup>17–19</sup> In those cases, the deletions always included more than one nucleotide each (ranging from 2 to 21 bp). A similar variant in the *AVPR2* gene, in which two insertions of single nucleotides (93insT and 96insT) introduce a stop codon, has been described.<sup>20</sup>

In addition to environmental factors, the effect of potential modifier genes (genotype) on the phenotypic variation should be seriously considered. An obvious candidate for a modifier is the *ABCR* gene, for three reasons: Independent mutations in *ABCR* and *ELOVL4* result in a similar (STGD) phenotype; *ABCR* function is directly dependent on the membrane lipid environment,<sup>21</sup> which we hypothesize, is determined in part by *ELOVL4* function; and, as we have previously shown, a 6q14-linked patient with adSTGD who is a heterozygous carrier of a variant *ABCR* allele exhibits more severe phenotype than his siblings.<sup>22</sup> Direct sequencing of the entire *ABCR* and peripheral *RDS* genes in patients from the K4175 pedigree failed to uncover any obvious disease-associated variants. To definitively confirm (or reject) this hypothesis, we are screening several individuals from all other families in which the autosomal

dominant macular dystrophy phenotype is caused by the *ELOVL4* mutation on the ABCR350 microarray, a recently established comprehensive screening tool containing all currently known (>350) *ABCR* alleles.<sup>23</sup> In general, there was no definite segregation of the various disease phenotypes within the K4175 family. For example, one individual diagnosed with butterfly-pattern dystrophy (III-7) had three affected children, one with similar pattern dystrophy and two with typical Stargardt macular changes. An affected parent with flecks could have an affected child without flecks, and an affected parent without flecks could have a child with flecks.

In summary, we have determined a new mutation in the *ELOVL4* gene, which segregates with an autosomal dominant macular dystrophy phenotype in a large independent pedigree. This finding not only confirms that mutations in *ELOVL4* are the cause of variable macular phenotypes, but also further illustrates the complexity of phenotypic expressions of the genetic variation in photoreceptor-specific membrane-associated proteins. Together with *ABCR*, the *ELOVL4* gene expands our knowledge of possible metabolic (and/or signaling) pathways in photoreceptor outer segment membranes.

### Acknowledgments

The authors thank the patients, their families, and their physicians, without whose cooperation this work would not have been possible, and Brith Otterud for linkage analysis.

### References

- Kaplan J, Gerber S, Larget-Piet D, et al. A gene for Stargardt's disease (fundus flavimaculatus) maps to the short arm of chromosome 1. *Nat Genet.* 1993;5:308–311.
- Allikmets R, Singh N, Sun H, et al. A photoreceptor cell-specific ATP-binding transporter gene (*ABCR*) is mutated in recessive Stargardt macular dystrophy. *Nat Genet.* 1997;15:236–246.
- Maugeri A, Klevering BJ, Rohrdchneider K, Blankenagel A, et al. Mutations in the *ABCA4* (*ABCR*) gene are the major cause of autosomal recessive cone-rod dystrophy. *Am J Hum Genet.* 2000;67:960–966.
- Martinez-Mir A, Paloma E, Allikmets R, et al. Retinitis pigmentosa caused by a homozygous mutation in the Stargardt disease gene *ABCR*. *Nat Genet.* 1998;18:11–12.
- Cremers FP, van de Pol DJ, van Driel M, et al. Autosomal recessive retinitis pigmentosa and cone-rod dystrophy caused by splice site mutations in the Stargardt's disease gene *ABCR*. *Hum Mol Genet.* 1998;7:355–362.
- Allikmets R, Shroyer NF, Singh N, et al. Mutation of the Stargardt disease gene (*ABCR*) in age-related macular degeneration. *Science.* 1997;277:1805–1807.
- Allikmets R. Further evidence for an association of *ABCR* alleles with age-related macular degeneration. The International *ABCR* Screening Consortium. *Am J Hum Genet.* 2000;67:487–491.
- Stone EM, Nichols BE, Kimura AE, Weingeist TA, Drack A, Sheffield VC. Clinical features of a Stargardt-like dominant progressive macular dystrophy with genetic linkage to chromosome 6q. *Arch Ophthalmol.* 1994;112:765–772.
- Kniazeva M, Chiang MF, Morgan B, et al. A new locus for autosomal dominant stargardt-like disease maps to chromosome 4. *Am J Hum Genet.* 1999;64:1394–1399.
- Zhang K, Kniazeva M, Han M, et al. A 5-bp deletion in *ELOVL4* is associated with two related forms of autosomal dominant macular dystrophy. *Nat Genet.* 2001;27:89–93.
- Ayyagari R, Zhang K, Hutchinson A, et al. Evaluation of the *ELOVL4* gene in patients with age-related macular degeneration. *Ophthalmic Genet.* In press.
- Gass JDM, ed. *Stereoscopic Atlas of Macular Diseases*. 4th ed. St. Louis: CV Mosby; 1997.
- Edwards AO, Miedziak A, Vrabec T, et al. Autosomal dominant Stargardt-like macular dystrophy. I: clinical characterization, longitudinal follow-up, and evidence for a common ancestry in families linked to chromosome 6q14. *Am J Ophthalmol.* 1999;127:426–435.

14. Donoso LA, Frost AT, Stone EM, et al. Autosomal dominant Stargardt-like macular dystrophy: founder effect and reassessment of genetic heterogeneity. *Arch Ophthalmol*. 2001;119:573-575.
15. Lagali PS, MacDonald IM, Griesinger IB, Chambers ML, Ayyagari R, Wong PW. Autosomal dominant Stargardt-like macular dystrophy: segregating in a large Canadian family. *Can J Ophthalmol*. 2000;35:314-324.
16. Griesinger IB, Sieving PA, Ayyagari R. Autosomal dominant macular atrophy at 6q14 excludes CORD7 and MCDR1/PBCRA loci. *Invest Ophthalmol Vis Sci*. 2000;41:248-255.
17. Castellvi-Bel S, Sheikavandi S, Telatar M, et al. New mutations, polymorphisms, and rare variants in the ATM gene detected by a novel SSCP strategy. *Hum Mutat*. 1999;14:156-162.
18. Nijbroek G, Sood S, McIntosh I, et al. Fifteen novel FBN1 mutations causing Marfan syndrome detected by heteroduplex analysis of genomic amplicons. *Am J Hum Genet*. 1995;57:8-21.
19. Ahmad W, Zlotogorski A, Panteleyev AA, et al. Genomic organization of the human hairless gene (HR) and identification of a mutation underlying congenital atrichia in an Arab Palestinian family. *Genomics*. 1999;56:141-148.
20. Rocha JL, Friedman E, Boson W, et al. Molecular analyses of the vasopressin type 2 receptor and aquaporin-2 genes in Brazilian kindreds with nephrogenic diabetes insipidus. *Hum Mutat*. 1999;14:233-239.
21. Ahn J, Wong JT, Molday RS. The effect of lipid environment and retinoids on the ATPase activity of ABCR, the photoreceptor ABC transporter responsible for Stargardt macular dystrophy. *J Biol Chem*. 2000;275:20399-20405.
22. Zhang K, Kniazeva M, Hutchinson A, Han M, Dean M, Allikmets R. The ABCR gene in recessive and dominant Stargardt diseases: a genetic pathway in macular degeneration. *Genomics*. 1999;60:234-237.
23. Allikmets R, Hutchinson A, Jaakson K, Kulm M, Pavel H. Genotyping microarray (gene chip) for the ABCR (ABCA4) gene. [ARVO Abstract]. *Invest Ophthalmol Vis Sci* 2001;42(4):S714. Abstract nr 3845.